

Molecular and Metabolic Mechanisms of Carbon Sequestration in Marine Thrombolites

Graduate Student Researcher: Jennifer Mobberley, Ph.D. candidate

NASA - John F. Kennedy Space Center

Graduate Student Researchers Program (GSRP) - Final Report

Award Date: 08/15/10 - 07/31/13

Expected Graduation: Fall 2013

NASA Project Number: NNX10AO18H

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Overview of Research Project: The overall goal of my dissertation project has been to examine the molecular processes underlying carbon sequestration in lithifying microbial ecosystems, known as thrombolitic mats, and assess their feasibility for use in bioregenerative life support systems. The results of my research and education efforts funded by the Graduate Student Researchers Program can be summarized in *four peer-reviewed research publication, one educational publication, two papers in preparation, and six research presentations at local and national science meetings* (see below for specific details).

Peer Reviewed Publications

Mobberley, J.M., Khodadad, C.L.M. Foster, J.S. (2013) Metabolic potential in cyanobacterial-dominated thrombolitic mats. *Photosynthesis Research* DOI 10.1007/s11120-013-9890-6.

Mobberley, J.M., Ortega, M.C., and J.S. Foster (2012) Comparative microbial diversity analyses of modern marine thrombolitic mats by barcoded pyrosequencing. *Environmental Microbiology*. 14:82-100.

Myshrall, K.L., **J.M. Mobberley**, S.J. Green, P.T. Visscher, S.A. Havemann, R.P. Reid and J.S. Foster (2010) Biogeochemical cycling and microbial diversity in the modern marine thrombolites of Highborne Cay, Bahamas. *Geobiology* 8:337-354.

Foster, J.S. and **J.M. Mobberley** (2010) Past, present, and future: microbial mats as models for astrobiological research. In: *Cellular Origin, Life in Extreme Habitats and Astrobiology: Microbial Mats: Modern and Ancient Microorganisms in Stratified Systems*. (J. Seckbach and A. Oren, eds.) Springer, pp. 563-582.

Educational Learning Module

Foster, J.S. and **J.M. Mobberley** (2011) Introduction to microbial diversity: a practical exercise. *Multimedia Educational Resource for Learning and Online Teaching* (MERLOT). www.merlot.org/merlot/viewMaterial.htm?id=571261.s

Publications in Preparation

Mobberley, J.M., Khodadad, C. L. M, Visscher, P.T, Rodriguez-R, L.M., Konstantinidis, K.T., Foster, J.S. (2013) Metatranscriptomic analysis of modern thrombolitic mats. *Proceedings National Academies of Science* (submission in August)

Mobberley, J.M., Louyakis, A.S., Visscher, P.V., and J.S. Foster (2013) Spatial differences in bacterial and archaeal diversity within a modern thrombolitic mat. *International Society of Microbial Ecology Journal* (submission in September)

Presentations of Research Results:

Mobberley, J.M. and J.S. Foster. Exploring the metabolic potential of thrombolitic mats from Highborne Cay, Bahamas. Florida Branch American Society of Microbiology annual meeting, Islamorada, FL, April 2013.

- Mobberley, J.M.,** Khodadad, C.M., and J.S. Foster. Assessing the functional gene complexity of modern thrombolitic mats using metagenomics and metatranscriptomics. International Society of Microbial Ecology Meeting-14, Copenhagen, DK, August 2012.
- Mobberley, J.M.,** Microbialitic mats as models for astrobiological research. International Space University Space Studies Program, Kennedy Space Center, FL, July 2012.
- Mobberley, J.M.,** Khodadad, C.M., and J.S. Foster. Functional genetic diversity of modern thrombolitic mats of Highborne Cay, Bahamas. Astrobiology Science Conference, Atlanta, GA, April 2012.
- Mobberley, J.M.** Assessing the functional gene complexity of modern thrombolitic mats using metatranscriptomics. University of Florida Department of Microbiology and Cell Science Seminar Series, April 2, 2012.
- Mobberley, J.M.** Exploring the functional complexity within thrombolitic mats. University of Florida Graduate Student Symposium, Jacksonville, FL, August 19, 2011.

Project Summary:

Bioregenerative life support systems (BLSS) that improve the recycling and sequestration of carbon dioxide (CO₂) are needed for long duration space flight. One ecosystem that has the potential to be used for BLSS are *thrombolitic microbial mat communities*. These microbial communities have been shown to undergo robust recycling of CO₂ and are highly resilient to environmental flux (Nelson, 2008). Thrombolitic mats are self-sustaining microbial communities that undergo extensive biologically induced precipitation of CO₂ as calcium carbonate (CaCO₃). Thrombolitic mats are ideal model systems for examining the mechanisms of carbon sequestration, as they function as semi-closed environments in which carbon is cycled with great efficiency (Canfield and DesMarais, 1993) and they have rates of productivity that are comparable to hardwood forests and grasslands (Canfield et al., 2004). One of the most well-studied thrombolitic mat ecosystems is found on the island of Highborne Cay, Bahamas. Microbial diversity and biogeochemical studies of these Bahamian thrombolitic mats revealed complex microbial consortia contributing to daily metabolic cycling of carbon (Myshrall et al., 2010; Mobberley et al., 2012). The net mat microbial metabolisms create biochemical gradients, such as pH and oxygen, which influence the extent of CaCO₃ precipitation (Visscher et al. 2005; Dupraz et al., 2009). There is a *significant gap in knowledge* of the functional genetic mechanisms that contribute to CO₂ sequestration within thrombolitic mats.

The *goal of my project* has been to understand the metabolic processes that contribute to community functioning and carbon sequestration within thrombolitic mat communities. The *overall objective* of this research plan was to assess and delineate functional gene expression of modern lithifying microbial mat ecosystems. The results of this research project are expected to have a *positive impact* on understanding the active role microbes play in coordinating metabolisms within thrombolitic mat communities and may also lead to the identification of novel metabolic pathways that could be utilized in future bioregenerative life support systems.

Undergraduate Training and Educational Outreach: In addition to my research training I also gained experience in mentoring and training others students in STEM education. Over the past three years I have participating in the following activities:

- Mentored two undergraduate interns working at the Space Life Sciences Lab with Dr. Jamie Foster. These students were Maya Ortega, a NASA MUST intern from University

of Colorado and Steven Ahrendt, a Florida Space Grant intern from University of California Riverside.

- Served as a science fair judge for the 2011 Brevard County Regional Science Fair.
- Assisted the NASA Kennedy Space Center Education Office by providing incoming interns with a tour of the University of Florida research facilities at the Space Life Science Lab.

Major Research Findings and Accomplishments as a result of GSRP funding:

• **Microbial diversity of modern marine thrombolites are distinct from other lithifying microbial mat ecosystems.** The results of my research indicated that there are four different thrombolitic mat types at Highborne Cay, The Bahamas. Additionally, my work revealed that the microbial diversity contained in the unlaminated thrombolitic mats of Highborne Cay is distinct from other lithifying microbial ecosystems. I examined the bacterial, cyanobacterial and archaeal populations within the various mat types. These results suggest that the unlaminated microstructure characteristic thrombolites may be formed by the metabolic activities of a distinct population of microbes dominated by the cyanobacterium *Dichothrix* spp. An example of the bacterial derived from the different thrombolitic mat types (i.e., black, beige, pink, and button mats) is seen in Figure 1.

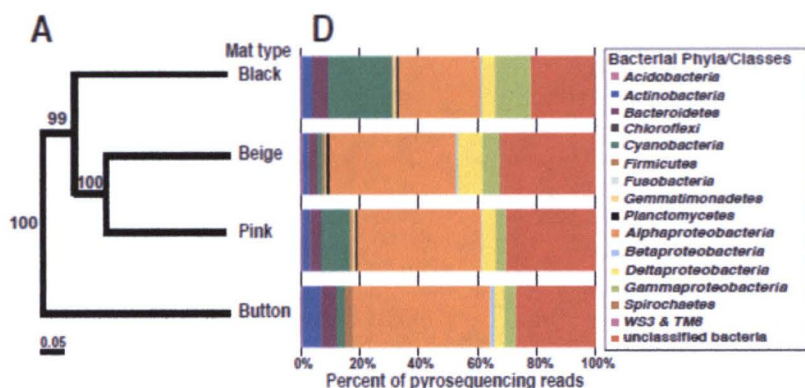


Figure 1. Comparisons of thrombolitic mats using both clustering-based and taxonomic diversity approaches. Dendrograms show UniFrac-based jackknife environmental clustering of the four thrombolitic mat types for bacterial (A). The percent of bootstrap replicates ($n = 1000$) are indicated at the nodes. The histograms (D) represent the relative abundance as the per cent of the total sequences classified into a specific taxon by a Bayesian approach at a confidence interval 70% against the SILVA SSU Ref v102 database with bacterial libraries. (taken from Mobberley et al., 2012)

• **Established the metabolic potential of thrombolitic mat through metagenomic sequencing.** The range of biochemical and molecular pathways that contribute to thrombolitic mat functional gene complexity were delineated through the sequencing of total community DNA extracted from the thrombolitic mats of Highborne Cay, Bahamas (Mobberley et al., 2013). The metagenome was annotated using the SEED genome database and sets of related genes involved in metabolic pathways (i.e., subsystems) were compared using the metagenome statistics program, R. The results indicated that the metagenomes of the thrombolitic mats are distinct from previously sequenced lithifying microbial ecosystems suggesting there are potentially unique processes associated with the carbonate structure formation (Figure 2). The metagenome was dominated by bacterial metabolisms, with overrepresented subsystems associated with Protein, Phosphorus, Nitrogen, Carbohydrate, Mobile Elements, and Amino Acid Metabolisms (Figure 3A). These results are the *first metagenomic analysis* of the Bahamian thrombolites and suggest that the microbes associated with these communities are capable of a wide range of metabolic activities that may influence carbon sequestration and carbonate mineralization.

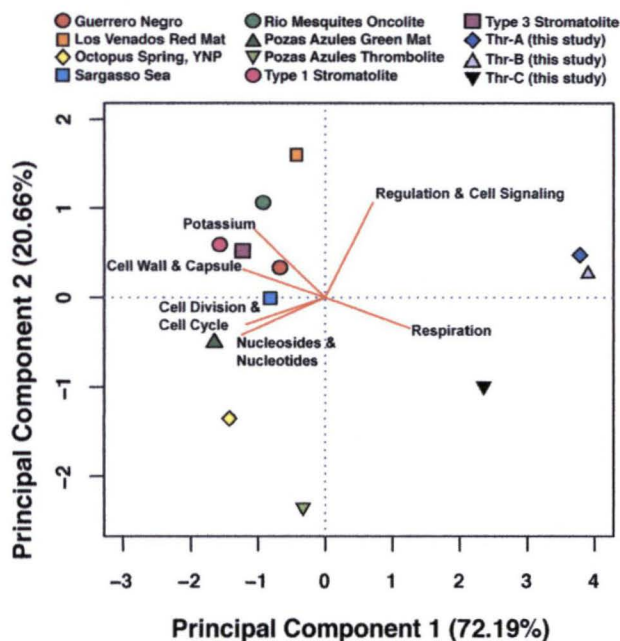


Figure 2 Comparison of thrombolite metagenome with metagenomes of previously sequenced lithifying and non-lithifying microbial mat ecosystems. a Principle Component Analysis of SEED subsystems (Level 1) derived from several distinct habitats. These habitats include the hypersaline non-lithifying mats of Guerrero Negro, Mexico; thermophilic microbial mats of Octopus Spring, Yellow Stone National Park (YNP); freshwater microbial mats of Los Venados and Pozas Azules found in Cuatros Cienegas Basin, Mexico (CCB); lithifying freshwater oncolites and thrombolites of Rio Mesquites and Pozas Azules in CCB; non-lithifying (Type 1) and lithifying (Type 3) stromatolites of Highborne Cay, The Bahamas and the unlaminated thrombolites of this study. A metagenome from the Sargasso Sea was used as an outgroup. The first two principle components represent 92.85 % of the variation between samples. The red biplot lines represent the directionality of the six most important subsystems driving differences between the metagenomes included in the analysis. (taken from Mobberley et al., 2013)

• **Generated first metatranscriptomic gene expression profile of modern marine thrombolites.**

To complement the metagenomic data, I also examined the metatranscriptome of the thrombolitic mats using direct transcriptome sequencing. Metatranscriptomics is the profiling of gene expression via the analysis of message RNA (mRNA) transcripts found in multispecies communities and has advanced our understanding of complex microbial ecosystems at the molecular level. To examine the metatranscriptome and generate a spatial profile of gene expression in the thrombolitic mats I horizontally sectioned the mats collected at the peak of solar activity (12PM) into three discrete zones (0 – 3 mm; 3 – 5 mm; and 5 – 9 mm) based on *in situ* biogeochemical measurements of oxygen production in the mats using a Clark microelectrode (Figure 4).

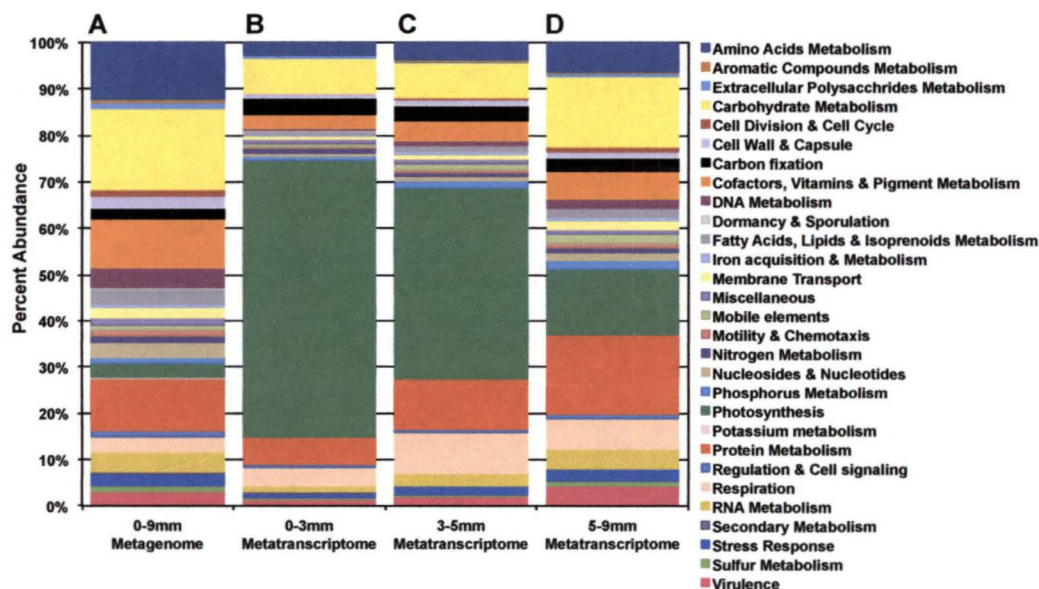


Figure 3. Distribution of recovered functional genes of the thrombolitic mats. All samples were collected at 12 PM. (A) Metagenome represents sequencing of community DNA extracted from the three combined layers. Metatranscriptomic libraries of total RNA from (B) Layer 1 (0 – 3 mm); (C) Layer 2 (3 – 5 mm); and (D) Layer 3 (5 – 9 mm).

I generated ~3 Gbp of metatranscriptomic sequencing data for each layer of the thrombolitic mat. For control purposes I also sequenced the total RNA to determine the efficiency of commercial mRNA purification kits. I determined that the mRNA enrichment step, which was intended to remove ribosomal RNA (rRNA), was not effective for microbial mat samples and sequencing total RNA not only minimized sequencing bias but was a faster and less expensive approach for future metatranscriptomics analyses. I also developed a metatranscriptome analysis pipeline using pre-existing software and custom Perl and Python scripts to classify potential functional genes from the recovered mRNA transcripts. Additionally, I analyzed the recovered rRNA sequences, which will serve as proxies for those metabolically active species within the thrombolitic mat. The results of the functional gene analyses are listed below.

• ***Metatranscriptomic sequencing revealed differential gene expression within thrombolitic mats.***

The annotated metatranscriptomic data from each of the three layers was compared to the metagenome to assess the differential expression of genes associated with key metabolic pathways (Figure 3). Not surprisingly key differences in the relative abundance of gene expression were detected between the metagenome and metatranscriptome in several metabolic pathways. The largest difference occurred in genes associated with photosynthesis.

Although photosynthesis comprised only 4% of the metagenome, between 13 and 60% of the recovered transcripts were photosynthetic genes. Taxonomic analysis indicated that most of the primary production was done by cyanobacterial oxygenic photosynthesis with some contribution from eukaryotic algae. These molecular results also correlated to the increase in oxygen production that occurred at those depths (Figure 4). These results provide further evidence that photosynthesis is a major metabolism of the thrombolitic mats and may be driving the biogeochemical cycling within the thrombolitic mat. Photosynthesis is also an important metabolism in carbonate mineralization (Dupraz et al., 2009), and the extensive up-regulation of photosynthesis pathways may be facilitating the precipitation of carbonate in the thrombolitic microbialites.

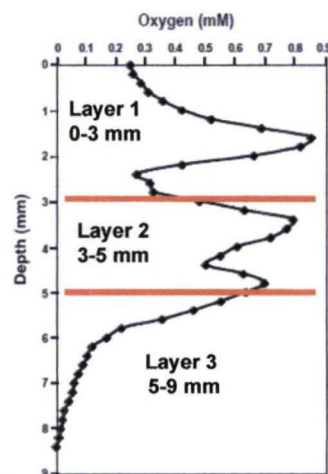


Figure 4. Representative oxygen depth profile of Bahamian thrombolitic mat generated using a Clark microelectrode at 12:00 pm, when the photosynthetic active radiation was at its peak ($1848 \mu\text{Em}^{-2}\text{s}^{-1}$). Profile reveals pronounced oxygen signature with multiple oxygen maximum and minima.

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